Docket No.12090-03USA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Hyun J. Hwang *et al.*

Application No.: 10/801,342 | Confirmation No.: 2316

Filed: March 15, 2004 | Art Unit: 1637

For: *METHOD AND APPARATUS FOR AMPLIFICATION OF NUCLEIC ACID SEQUENCES BY USING THERMAL CONVECTION* | Examiner: Pande, Suchira

SECOND DECLARATION PURSUANT TO 37 CFR 1.131

The undersigned declare as follows:

1. We are co-inventors of the above-identified application (hereinafter «subject application»). In particular, Hyun Jin Hwang is Chairman and Chief Executive Officer of Ahram Biosystems, Inc. of Seoul, Korea; Jeong Hee Kim is Professor at the Kyung Hee University of Seoul, Korea as well as Scientific Advisor of Ahram Biosystems, Inc. of Seoul, Korea; and Kyunghoon Jeong is R&D Manager at Young Lin Instrument Co., Ltd. of Anyang, Korea.

2. The subject application discloses, among other things, an apparatus and method for amplifying nucleic acid sequences. The subject application discloses, for example, an apparatus with a plurality of spatially defined heat sources that supply or remove heat from a sample and create thermal convection conditions for amplifying the nucleic acid sequences.

3. The invention described and claimed in the subject application was conceived and reduced to practice in the Republic of Korea prior to October 5, 2000.

4. For instance, and well before October 5, 2000, at least one of us recognized the need to make a nucleic acid sequence amplification apparatus as described in the subject application that

uses a convection-based polymerase chain reaction (PCR) to amplify nucleic acid sequences. Attached as **Appendix 1** is a true and accurate copy of a photograph of the apparatus (dissembled), that shows, among other things, *a plurality of heat sources which supply heat to or remove heat from a plurality of specific regions in a sample*. For example, a first heat source with sample hole depths of 5 mm, 10 mm, and 15 mm (see test tubes at right), a second heat source (left), and an insulator positioned between the first and second heat sources (center, with connecting bolts).

5. The nucleic acid sequence amplification apparatus made by at least one of us well before October 5, 2000, is designed so that *the plurality of heat sources are arranged such that a first heat source providing heat to a lower portion of the sample is located lower in height than a second heat source removing heat from an upper portion of the sample*. Attached is **Appendix 2** which shows, among other things, the apparatus (assembled) so that the first heat source (bottom) is located lower in height than the second heat source (top) with regard to the sample.

6. The **Appendices 1 and 2** further show, among other things, recognition by at least one of us well before October 5, 2000 that there was a need to vary sample hole depth in the first heat source to depths 5, 10 and 15 mm (see the test tubes as located at different depths), respectively, to create a depth sufficient to provide *a spatial temperature distribution with spatial regions fulfilling temperature conditions suitable for convection PCR* in the apparatus.

7. The **Appendix 1** further shows, among other things, that well before October 5, 2000, we made the nucleic acid sequence amplification apparatus to include *an insulator to be positioned between the first and second heat sources* (center, with connecting bolts), and the first heat source with sample hole depths of 5 mm, 10 mm and 15 mm (right).

8. The **Appendices 1 and 2** also show, among other things, that well before October 5, 2000 at least one of us recognized the need to include a copper inlet tube and a copper outlet tube in the second heat source (left). These components allowed *a liquid* such as water to be *in thermal contact with a specific region of the sample* in contact with the second heat source. As further shown in **Appendix 3** (below), the second heat source was further made before October 5,

2000 to include a *receptor* (embedded between the sample tube holes inside the second heat source) *in which the liquid was contained to remove heat from the sample*. Depending on the experiment, the temperature of the liquid water flowing through the copper inlet and outlet tubes was readily controlled by at least one of us so that the second heat source could heat or cool the sample or the sample tube as needed.

9. **Appendix 3** is a true and accurate copy with dates and irrelevant information removed of a schematic drawing made by at least one of us (or by another under our direction and supervision) well before October 5, 2000 that shows, among other things, a horizontal view of the second heat source of the apparatus. The drawing shows, among other things, the receptor (shown with dotted lines) for containing the liquid, embedded between the sample tube holes within the second heat source of the apparatus. In the drawing, «A» indicates a drilled hole that was sealed by soldering with a copper rod disk after the drilling and «B» indicates a drilled hole that was soldered to a copper tubing after the drilling to provide the copper inlet and outlet tubes. The inlet and outlet tubes and receptor of the second heat source shown in **Appendices 1-2,** for example, were designed by at least one of us to produce or assist *a circulation unit that would circulate liquid in the apparatus*.

10. The **Appendices 1 and 2** also illustrate, among other things, that well before October 5, 2000, the nucleic acid amplification apparatus by at least one of us included *a plurality of heat sources with a first thermally conductive solid in thermal contact with a lower portion of the sample and a second thermally conductive solid in thermal contact with an upper portion of the sample*. In the apparatus shown in the appendices, the first and second thermally conductive solids include copper to facilitate heat transfer.

11. **Appendices 1** and **2** further show, among other things, that well before October 5, 2000 at least one of us conceived and made a nucleic acid amplification apparatus in which *at least one of the heat sources includes a thermally conductive solid in thermal contact with a specific region of the sample*. See, for example, **Appendix 1** (particularly the two heating blocks on the left and right side which include sample tube holes).

12. The **Appendix 1** also shows, among other things, that well before October 5, 2000, the apparatus conceived and made by us included *an opening defined by the first and second heat sources and the insulator*. The opening is adapted to receive a reaction vessel with sample. Referring again to **Appendix 1**, the apparatus has nine (9) of such openings that are defined by the second heat source (left), the insulator (middle) and the first heat source (right). Three (3) reaction vessels received by the openings in the apparatus are shown (right).

13. The **Appendix 1** also shows, among other things, that well before October 5, 2000, the opening for receiving the reaction vessels included *a first through hole within the second heat source* (left), *a second through hole within the insulator* (middle) and an opening within the first heat source (right). *The opening is essentially perpendicular to the insulator* and *it is adapted to receive a reaction vessel configured as a straight cylinder or tube* (3 such tubes are shown, right).

14. **Appendix 4** is a true and accurate copy with dates and irrelevant information removed of a schematic drawing made by at least one of us (or by another under our direction and supervision) well before October 5, 2000 that shows, among other things, a vertical section taken through certain sample tube holes of the first heat source to show *a closed bottom end within the first heat source*. The plane of section was taken through sample tube holes of equal depth within the apparatus.

15. Well before October 5, 2000, at least one of us prepared a Report with reaction conditions to test the apparatus under convection PCR conditions. **Appendix 5** is a true and accurate copy, with dates and irrelevant information removed, that shows, among other things, reaction conditions designed by at least one of us for confirming that the apparatus could amplify nucleic acid under convection PCR conditions. In particular, the Report shows, for instance, data from an experiment in which a single-stranded (ss) 65 oligonucleotide template (65-mer), amplifying primers (KS, SK), dNTP mix, buffer, $MgCl_2$, TAQ enzyme and volume parameters are specified (Report, part 1). Further convection PCR reaction conditions for the experiment as shown in the **Appendix 5** were: a temperature for heating (95°C-104°C), for cooling (around 50° C), a reaction volume of 70 microliters, and a reaction time of 3 hours.

16. In the Report shown in the **Appendix 5**, reference to "Convection PCR" means convection PCR in solution as described throughout the subject application.

17. The **Appendix 5** also shows, among other things, that well before October 5, 2000 at least one of us used the apparatus to perform convection PCR and amplify the 65-mer template. In particular, the bottom portion of **Appendix 5** is a photograph of a stained agarose gel showing amplification of the template in two lanes to the right of the lane with DNA size standards (furthest lane to left). At least one of us determined from this gel that successful convection PCR amplification was achieved under the reaction conditions specified in the Report when the apparatus had a sample tube hole depth of 15 mm in the first heat source (13 mm from the bottom of the sample).

18. At least one of us concluded from the data presented in **Appendix 5**, for instance, that the apparatus we made and tested well before October 5, 2000, could produce *a spatial temperature distribution with spatial regions fulfilling temperature conditions suitable for i) a denaturation step in which double strand DNAs become separated to single strand DNAs .* Referring again to **Appendix 5**, at least one of us concluded that the denaturation step could occur, for instance, at or near the bottom of the glass tube within the first heat source having the 95°C-104°C reaction temperature, for instance, at a depth of between 0 mm to about 10 mm or less in the sample tube used for the experiment shown.

19. At least one of us further concluded from the data presented in **Appendix 5,** for instance, that the apparatus we made and tested well before October 5, 2000 could produce *a spatial temperature distribution with spatial regions fulfilling temperature conditions suitable for (ii) an annealing step in which the single strand DNAs formed in the denaturation step hybridize to the primers to form DNA-primer complexes.* Referring again to **Appendix 5**, the annealing step could occur, for instance, at an upper region of the glass tube within the second heat source, for instance, between about 20 mm to about 30 mm or less from the bottom end of the sample tube of the experiment provided.

20. At least one of us further concluded from the data presented in **Appendix 5**, for example, that the apparatus we made and used well before October 5, 2000 could produce *a spatial temperature distribution with spatial regions fulfilling temperature conditions suitable for (iii) a polymerization step in which the primers in the DNA-primer complexes are extended by the polymerization reaction.* Referring again to **Appendix 5**, at least one of us concluded that the polymerization step could occur, for example, at an intermediate region of the glass tube between the denaturation region in the first heat source and the annealing region in the second heat source, for instance, between from about 10 mm to about 20 mm from the bottom end of the sample tube used for the experiment shown.

21. At least one of us further concluded from the data presented in **Appendix 5**, for instance, that *the heat sources* of the apparatus we made and used well before October 5, 2000, *were arranged to provide for a spatial temperature distribution that included a convection region positioned between a relatively high temperature region and a relatively low temperature region*. Referring to the data presented in **Appendix 5,** at least one of us concluded from the spatial temperature distribution data that the convection region was position between the high temperature region in the first heat source and the low temperature region in the second heat source within the sample tube. For instance, between from 0 mm to about 10 mm (high temperature region) and from about 20 mm to about 30 mm or less (low temperature region) from the bottom of the sample tube used in the experiment provided.

22. Well before October 5, 2000, at least one of us conceived of a reaction vessel configured to fit within the apparatus we made and tested well before that date. For instance, *a reaction vessel with a single passage between the relatively high temperature region and the relatively low temperature region* was used in our apparatus. The **Appendix 1** shows, among other things, three reaction vessels placed within the apparatus having an outer diameter (Φ) of 7.9 mm, and an inner diameter (Φ) of 2 mm with vessel wall thickness of 3 mm (top right).

23. In particular, and well before October 5, 2000, at least one of us concluded from the information presented in the **Appendices 1, 2, and 5** for example, that the single passage of the reaction vessel is adapted to contain the convective flow (upward and downward) that we

detected and identified within our apparatus when used. We further concluded from this information that, among other things, a reaction vessel with a single passage used in our apparatus achieved nucleic acid amplification through bidirectional convection (i.e., both upward and downward convection) within the reaction vessel as explained throughout the subject application.

24. The **Appendices 1 and 2,** for instance, further show, among other things, a reaction vessel positioned vertically with respect to the heat sources. Also shown is a reaction vessel suitable for use with the apparatus with a top and bottom end in which the bottom end is closed.

25. The **Appendices 1 and 2,** further show, among other things, that the apparatus made and tested well before October 5, 2000 could accommodate multiple reaction vessels.

26. The **Appendix 2** further shows that well before October 5, 2000 at least one of us conceived of the need to introduce a vertical gap between the top of the relatively high temperature region and the bottom of the relatively low temperature region. See the vertical gap (just visible) dividing the first and second heat sources and comprising the insulator shown in **Appendix 1**. The vertical gap is shown as a solid (insulator) in **Appendix 1** (middle). The apparatus shown in **Appendix 1** also provides for a gap between the sample and at least the second heat source.

27. The **Appendices 1 and 2** further show that well before October 5, 2000, at least one of us conceived of the need to make a nucleic sequence amplification apparatus in which the insulator is air. In particular, at least one of us recognized that the apparatus could be assembled for some experiments by joining the second heat source (left) and the first heat source (right) with the four connecting bolts (center) such that an insulating air gap was formed between the first and second heat sources.

28. We hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful statements and the like so made are

punishable by fine or imprisonment, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 29 MAY 2009 Name: Hyun Jin Hwang

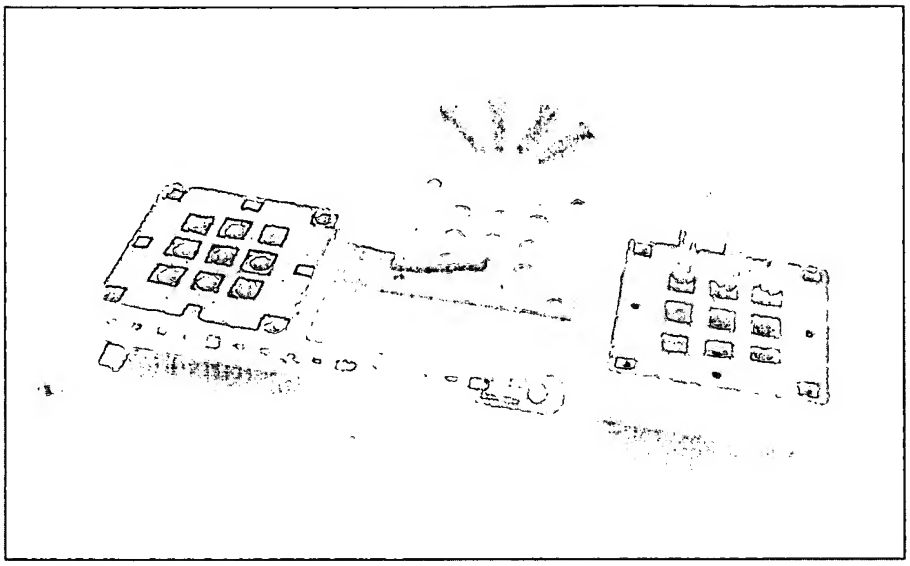
Hyun Jin Hwang

Date: 29 May 2009 Name: Jeong Hee Kim

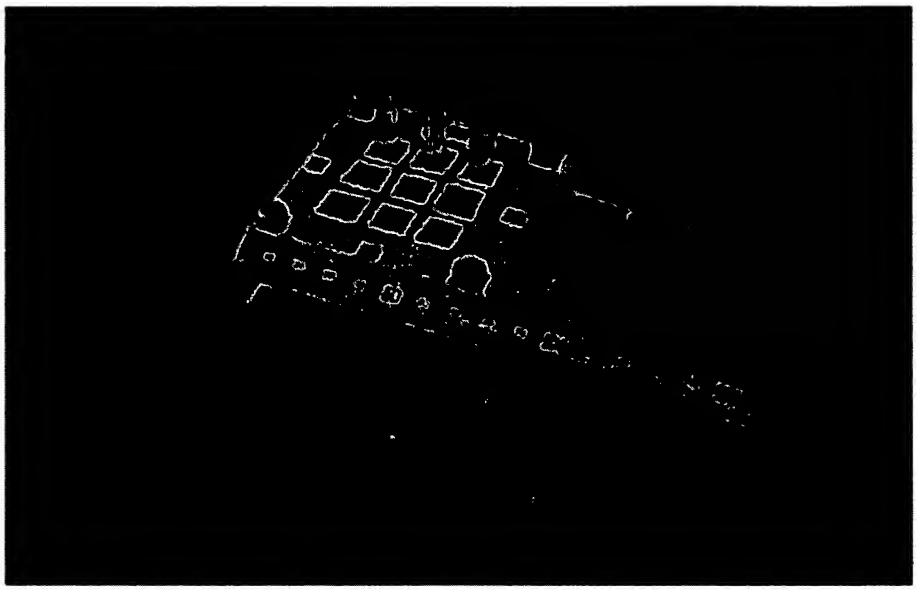
Jeong Hee Kim

Date: 29 May 2009 Name:

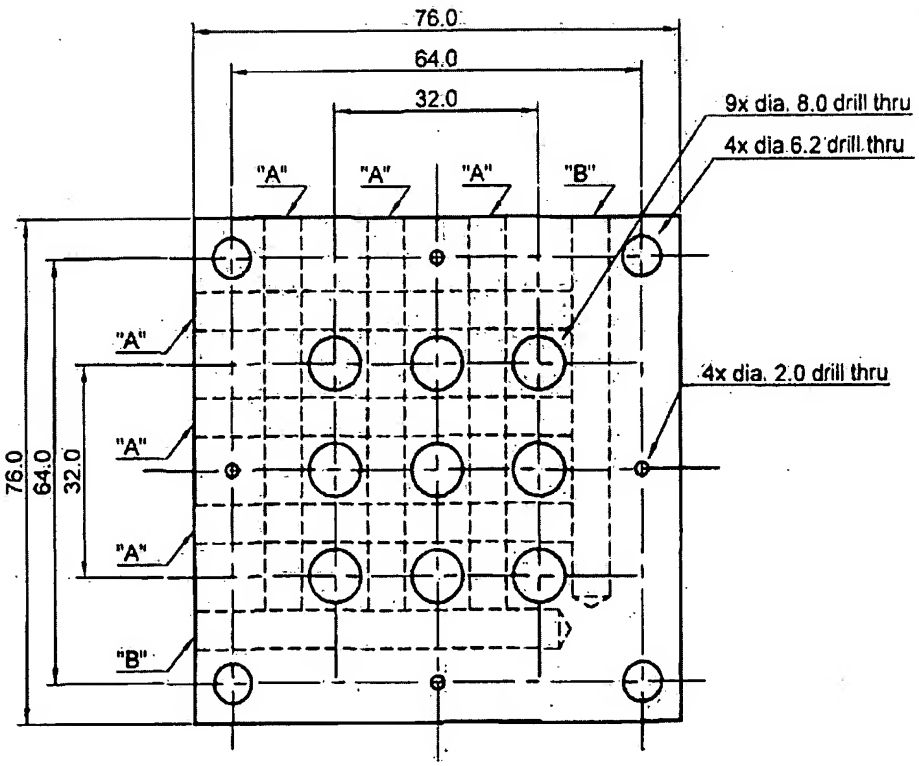
Kyunghoon Jeong



Appendix 1



Appendix 2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"A"
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"A"
"B"
4x dia. 2.0 drill thru
76.0
64.0
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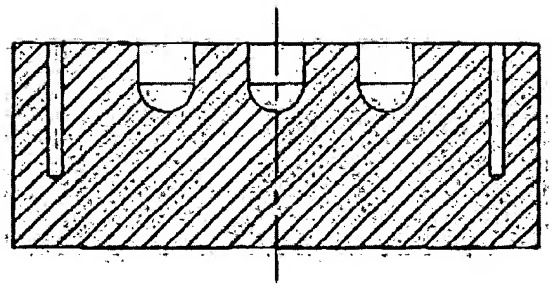
Appendix 3



Appendix 4

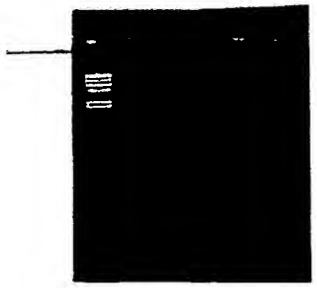
○ Convection PCR

1. Preparation of PCR solution

65mer (ss-DNA)	10 nM	35 fmol	3.5 ㎕
KS primer	2 ㎛M	14 pmol	7 ㎕
SK primer	2 ㎛M	14 pmol	7 ㎕
dNTP mix	0.25 mM	1.4 nmol	5.6 ㎕
10X buffer			7 ㎕
$MgCl_2$	25 mM	175 nmol	7 ㎕
DI-Water			32.9 ㎕
Total			70 ㎕

TAQ	1.06 ㎛M	1 monolayers for 3x5 ㎟ Au	0.371 pmol	0.35 ㎕

2. PCR temperature and time control
   - Put 70 ㎕ solution into glass tube .
   - Hot start step for 10 min at 94℃- activating taq in dry bath..
   - The bottom of glass tube is at 95~104℃.
   - Reaction for 3 hours.
   - Keep at 72℃ for 10 min, and cool down to 4℃.



Appendix 5